Journal of Agroforestry and Environment

Volume 17, Issue 2, 2024

Journal DOI: https://doi.org/10.55706/jae
Journal homepage: www.jagroforenviron.com

Efficacy of Indigenous Botanicals for Controlling Rice Weevil, *Sitophilus oryzae* L.

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Received: 14/09/2024

Accepted: 27/12/2024

Available online: 31/12/2024

Abstract: Significant losses and damage have been incurred in rice storage due to rice weevils (*S. oryzae*). Chemical pesticides have been used to control them, but this has had detrimental impacts on the environment and limited storage facilities. The n-hexane, dichloromethane (DCM) and methanol extracts of leaf and seed/fruit of karanja, *Pongamia pinnata* (L.); mahogany, *Swietenia mahogani* Jacq.; neem, *Azadirachta indica* A. Juss and urmoi, *Sapium indicum* Willd. at 2.0, 4.0, 6.0, 8.0 and 10.0% (w/v) concentrations were evaluated for their insecticidal effect against rice weevil, *Sitophilus oryzae*. Among the test plants, the highest mortality percentage was observed in urmoi (47.00%) and neem (44.22 %), respectively at 72 HAT. Among the solvents, dichloromethane extract showed more toxicity (mortality 52.67%) than other extracts. The effectiveness of most of the plant extracts were found to increase proportionately with the increase of doses. Mortality percentage increased with the progress of time. Among four plant extracts, the LC_{50} values (0.72%) at 72 HAT indicated that the urmoi extract also maintained its highest toxicity, followed by neem extract (LC_{50} values 0.91%). Thus, urmoi plant extracts can be utilized as botanical pesticides to control infestations of rice weevil.

Keywords: Plant extract; Toxicant effect; Rice weevil.

INTRODUCTION

Rice is a widely consumed cereal grain and is the staple food for many people in Asian countries. The rice weevil (*S. oryzae* L.) is one of the most widespread and destructive major insect pests of stored product throughout the world (Plague *et al.*, 2010; Khani *et al.*, 2011). Both the adults and larvae feed on different stored cereal grains viz. rice, wheat, maize and sorghum products causing serious losses, particularly in the monsoon. Under favourable prolonged storage conditions, it can losses up to 80%, whereas average losses between 10-65% are reported under moderate storage conditions (Park *et.al.* 2004).

To control the infestation of rice weevils, several types of chemical pesticides and insecticides such as methyl bromide and phosphine have been widely used in the storage of grain and processing plants. However, the negative effects of methyl bromide and the limitations of phosphine have been discovered (Negahban *et. al.*, 2006).

The needs to find new model molecules that facilitate the development of less poisonous and environment friendly materials for stored-product protection have stimulated research into the insecticidal properties of plant natural products (Grainge and Ahmed, 1988). Toxicants are specific types of chemicals, which directly kill insects. They are also referred to as insecticides. Worldwide reports on the toxicity of different plant derivatives showed that many plant products are toxic to stored - product insects (Islam and Talukder, 2005; Isman, 2006; Rajendran and Sriranjini, 2008). *Swietenia mahogany* a deciduous and economically important timber tree commonly known as ''mahogani,'' belongs to the family Meliaceae. The antifeedant activity of the limonoids from this plant has been reported recently (Abdelgaleil *et al.*, 2006). *Sapium indicum* (Euphorbiaceae), commonly known as urmoi (Bengali), is a small, semi-deciduous to evergreen tree. *S. indicum* is an Indian poisonous plant and piscicidal agent, and the unripe fruit contains an unusual metabolite which is an irritant (Taylor *et al.*, 1981).

The aim of this study was to evaluate the toxicant effect of n-hexane, dichloromethane (DCM) and methanol extracts (karanja, *Pongamia pinnata* L.; mahogany, *Swietenia mahogani* Jacq.; neem, *Azadirachta indica* A. Juss and urmoi, *Sapium indicum* Willd) on rice weevil for the development of botanical insecticides.

MATERIALS AND METHODS

Insect rearing

The rice weevil was collected from the stock culture of the Department of Entomology, Bangladesh Agricultural University, Mymensingh. The rice weevil was reared in round plastic jars (12 x 23 x 6.5cm in size) with rice grains (13 to 14% moisture) in growth chamber at 28±5°C temperature and R.H. 75±5% in the Entomology laboratory of BINA.

Plate 1**.** Rearing of rice weevil.

Collection and preparation of Plant sample

Fresh leaves and fruits of karanja *(Pongamia pinnata* L.); mahogany, *Swietenia mahogani* Jacq. and neem, *A. indica* A. Juss were collected from Bangladesh Agricultural University campus, Mymensingh. Leaves and fruits of urmoi, *Sapium indicum* Willd. were collected from Chalna under the District of Khulna.

Plate 2. Karanja branch

Plate 3**.** Neem branch

Plate 4**.** Mahogany branch

Plate 5. Urmoi plant

After collection, all fresh leaves of the test plants were washed with water and kept in the shade up to 15 days for air-drying. Mature seed(s) were collected from fresh fruits of karanja, mahogany, neem and mature fruits were collected from urmoi plant. The dried plant materials were then ground separately with electrical grinder and sieving through 60 micron sieve to obtain fine powder. The powder was preserved into plastic pot at low temperature (4˚C) till their use in extract preparation.

Extraction

Prepared leaf, seed and fruit powder were used for preparation of plant extract following the method of Tikum *et al.* (2008). The dried plant powders (50 g) were taken into a 400 ml beaker. The powder of leaves, seeds and fruits were extracted with n-hexane, dichloromethene and methanol. The extract was collected after 24 hours, filtered by fine cloth and concentrated by a rotary vacuum evaporator. The residual solvent was removed by high vacuum pump. Each of the extract was stored in a freezer until use.

Plate 6. Powder of plants materials

Plate 7. Extraction

Preparation of different doses

Plant extract stock solutions (20% w/v) were made by diluting the condensed extracts with the appropriate solvent. The plant extracts were dissolved in the same solvent to create various concentrations (2.0, 4.0, 6.0, 8.0, and 10.0% w/v) before the insect bioassay.

Plate 8. Plant extract concentrated with evaporator

Plate 9. Preparation of stock solution

Toxicant effect on rice weevil

The experiment was conducted according to the standard method number 1 described by McDonald *et al.* (1970) and modified by Talukder and Howse (1995). The adult insects were chilled for a period of 10 minutes. Then the immobilized insects were picked up individually by using a camel hair brush. One µl solutions of different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) of different extracts were applied to the dorsal surface of the thorax of each adult weevil using micro-pipette

Thirty adult weevils (1-2 weeks old) in three replicates of 10 insects (5♂ + 5♀) each, was treated at each dose. In addition, the same numbers were treated with solvent only as control. After treatment, the 10 weevils were transferred

DOI: https://doi.org/10.55706/jae1734

into 9 cm diameter Petri dish containing food. Adult weevils were examined daily and those that did not move or respond to gentle touch were considered as dead. Rice weevil adult mortalities were recorded at 24, 48 and 72 hours after treatment (HAT).

The observed mortality was corrected by Abbott's formula (1987):

Corrected mortality (%) = (Observed mortality–Control mortality/100 - Control mortality) x 100.

Statistics analysis

The data were analysed by ANOVA with CRD 4 Factor and significant mean values were compared with DMRT (Duncan, 1951). The per cent mortality data were transformed into arcsine values before ANOVA. The mortality data were used to determine LD_{50}. Then it was analysed by probit analysis using MSTAT-C Statistical Software in a computer which is based on the method of Finney (1971). The probit regression equations and lines were calculated for the relationship between probit mortality of insects and log doses of different plant extracts.

RESULTS AND DISCUSSION

The percentage of rice weevils that died at 24, 48, and 72 hours after treatment (HAT) showed that urmoi extract had the maximum toxic effect (mortality: 47.00%), whereas mahogany extract had the lowest toxic effect (mortality: 35.56%) at 72 HAT (Table 1). Mortality percentages were directly proportional to the time after treatment. The order of toxicity of four plant extracts on rice weevil was: urmoi > neem > karanja> mahogany.

Interaction of solvent and time

The 72 HAT value indicated that the highest mortality of rice weevil was found in the dichloromethane extract of urmoi (57.33%) and the lowest was recorded in the methanol extract of mahogany (26.67%) (Table 2). Among three solvents, dichloromethane extract was found to be significantly more toxic (52.67%) than other solvents at 72 HAT (Figure 1).

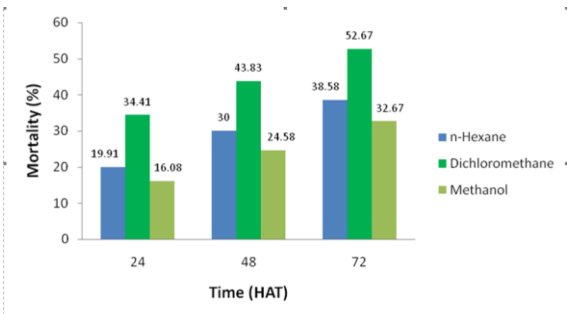

Figure 1. Effect of different plant extracts of different solvents on the mortality percentage of rice weevil by topical application method at different HAT

Interaction of dose and time

The percentage of insect mortality at 24, 48 and 72 hours after treatment indicated that mortality percentage was directly proportional to the level of doses and time after treatment (Figure 2). It was found that the interaction effect of dose and time after treatment with different plant extracts were significant at 1% level. Mean highest toxic effect of different plant extracts was found in 10% doses (57.22% mortality) at 72 HAT.

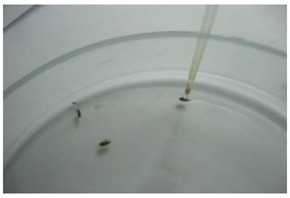

Plate 10. Toxicity test

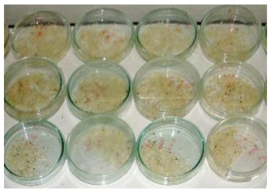

Plate 11. Experiment of toxicity effect

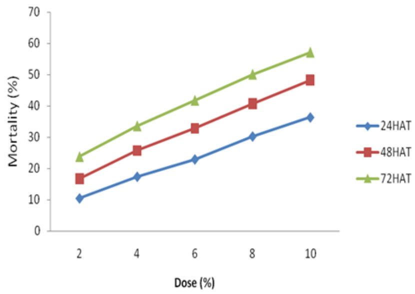

Figure 2. Mean effect of plant extracts at different dose level on the mortality of rice weevil by topical application method at different HAT

All the plant extracts had moderate toxic effect against rice weevil. Islam *et al.* (2001) reported that urmoi (*S. indicum*) extracts were more toxic than bitter gourd and mehedi extracts and that the mortality increased with the increasing concentration and exposure period. The present study also showed that fruit extracts of urmoi had strong toxic effect against rice weevil. The adult mortality is probably due to the presence of bioactive chemical components in plant products. The findings of Shahjahan *et al.* (2003), Rahman *et al.* (2004), Saha (2006), Begum *at al.* (2007), Khanam *et al.* (2008) and Mamun *et al.* (2009) also obtained similar results from their experiments.

Probit Analysis for Direct Toxic Effect

The results of the probit analysis for the estimation of LC_{50} values and their 95% fiducial limits and the slope of regression lines at 24, 48 and 72 HAT for the mortality of rice weevil have been presented in Table 3. The LC_{50} values of karanja (2.97%), mahogany (1.37%), neem (1.79%) and urmoi (1.43%) at 24 HAT indicated that

DOI: https://doi.org/10.55706/jae1734

mahogany was the most toxic followed by urmoi. At 48 HAT, comparison of LC_{50} values showed that urmoi extract (0.97%) was highly toxic and it was followed by neem extract (1.19%). Among four plant extracts, LC_{50} values at 72 HAT indicated that the urmoi extract (0.72%) was also maintained its highest toxicity followed by neem extract (0.91%) (Table 3). From the above probit results, it was clear that all the tested plants would be more or less effective for controlling rice weevil but urmoi and neem would be more effective. The chi-square values of different plant extracts at different HAT were insignificant at 5% level of probability except values with star mark (*) and did not show any heterogeneity of the mortality data.

Proboit Regression Lines

The probit regression lines for the effect of four different plant extracts (karanja, mahogany, neem and urmoi) are presented in Figures 3 – 5 for rice weevil. The calculated probit regression equations of four plant extracts for the lines at 24 HAT were: Y=4.325 + 1.412x, Y=4.622 + 2.7204x, Y=4.582 + 1.590x and Y=4.643 + 2.177x for karanja**,** mahogany**,** neem and urmoi, respectively (Figure 3).

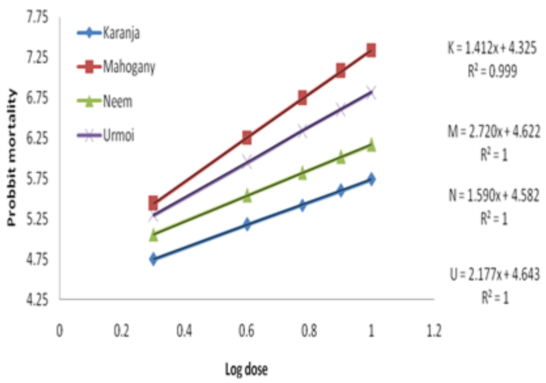

Figure 3. Relationship between log doses and probit mortality for four plant extracts against rice weevil at 24 HAT

Comparing among four lines, mahogany showed the highest probit mortality and karanja showed the lowest probit mortality. The calculated probit regression equations for the lines of four different plant extracts at 48 HAT were: Y=4.622 + 1.489x, Y=4.641 + 1.990x, Y=4.870 + 1.559 x and Y=5.028 + 2.130x for karanja**,** mahogany**,** neem and urmoi, respectively (Figure 4).

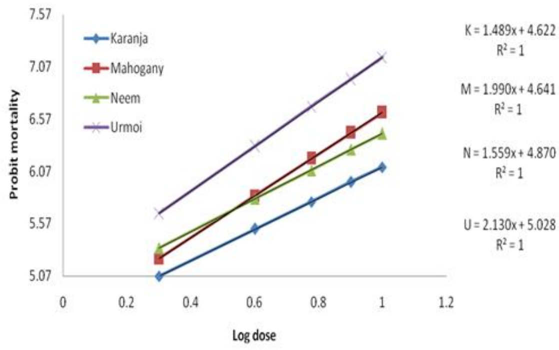

Figure 4. Relationship between log doses and probit mortality for four plant extracts against rice weevil at 48 HAT.

In this case, the highest and the lowest probit mortality were found with the urmoi and karanja plant extracts, respectively. At 72 HAT, the probit regression equations were calculated as Y=4.853 + 1.520x, Y=4.849 + 1.731x, Y=5.061 + 1.616x, and Y=5.299 + 2.260x for karanja**,** mahogany**,** neem and urmoi, respectively (Figure 5). Among the four lines, the regression line for urmoi plant extract also showed the highest and karanja showed the lowest probit mortality.

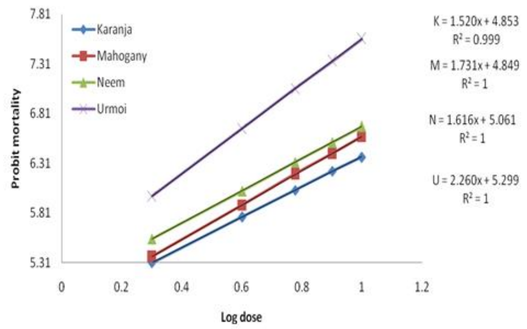

Figure 5. Relationship between log doses and probit mortality for four plant extracts against rice weevil at 72HAT

Comparing among four probit regression lines at 24 HAT, mahogany showed the highest probit mortality and karanja showed the lowest probit mortality (Figure 3). In case of 48 HAT, the highest and the lowest probit mortality were found with the urmoi and karanja plant extracts, respectively (Figure 4). Among the four lines at 72 HAT, the regression line for urmoi plant extract also showed the highest and kranja showed the lowest probit mortality (Figure 5). The insect mortality rate showed positive correlation with doses in all cases. The probit regression lines for the effects of four different plant extracts on rice weevil showed a clear linear relationship between probit mortality and their log doses.

DOI: https://doi.org/10.55706/jae1734

Table 1. Mean mortality percentage of rice weevil treated with extracts of different plants by topical application method at different HAT.

Name of the plant extracts	Mortality percentage		
	24 HAT	**48 HAT**	**72 HAT**
Karanja	20.89 b (23.78)	29.22 b (30.98)	38.44 b (37.59)
Mahogany	20.67b (24.13)	28.00 b (30.14)	35.56 b (35.78)
Neem	26.44 a (28.92)	36.00 a (36.22)	44.22 a (41.45)
Urmoi	25.89 a (28.82)	38.00 a (37.54)	47.00 a (43.64)
$S\bar{X}$	1.1493	0.9766	1.0289
Probability level	0.01	0.01	0.01

HAT= Hour after treatment.
Within column values followed by different letter(s) are significantly different by DMRT.
Figures in the parentheses are Arcsin transformed values.

Table 2. Mean mortality percentage of rice weevil treated with different plant extracts of different solvents by topical application method at different HAT.

Name of the plant extracts	Name of the solvents	Mortality percentage		
		24 HAT	**48HAT**	**72HAT**
Karanja	n-Hexane	14.33 f (17.54)	24.00 d (27.36)	31.67 de (33.32)
	DCM	35.33 ab (35.56)	42.67 ab (40.11)	56.00 a (48.50)
	Methanol	13.00 f (18.28)	21.00 d (25.47)	27.67 e (30.93)
Mahogany	n-Hexane	18.00 d-f (21.66)	26.33 d (28.86)	36.00 cd (36.28)
	DCM	28.33 bc (31.06)	36.67 bc (36.80)	44.00 b (41.24)
	Methanol	15.67 ef (19.67)	21.00 d (24.77)	26.67 e (29.81)
Neem	n-Hexane	25.00 cd (28.27)	36.67 bc (36.67)	46.33 b (42.75)
	DCM	41.33 a (38.43)	48.67 a (44.08)	53.33 a (47.01)
	Methanol	13.00 f (20.06)	22.67 d (27.88)	33.00 de (34.61)
Urmoi	n-Hexane	22.33 c-f (26.22)	33.00 c (34.48)	40.33 bc (39.16)
	DCM	32.67 b (33.74)	47.33 a (43.25)	57.33 a (50.87)
	Methanol	22.67 c-e (26.51)	33.67 c (34.88)	43.33 b (40.88)
$S\bar{X}$		1.9907	1.6915	1.7829
Probability level		0.01	0.01	0.01

HAT= Hour after treatment.
DCM=Dichloromethane.
Within column values followed by different letter(s) are significantly different by DMRT.
Figures in the parentheses are Arcsin transformed values.

DOI: https://doi.org/10.55706/jae1734

Table 3. Relative toxicity (by probit analysis) of four plant extracts treated against rice weevil at 24, 48 and 72 HAT.

Name of the plant extracts	No. of insect used	LC_{50} value (%)	95% fiducial limits	χ2 value	Slope ± SE
			24 HAT		
Karanja	900	2.97	1.51-5.80	1.95	1.41±0.01
Mahogany	900	1.37	1.14-1.64	7.38	2.72±0.01
Neem	900	1.79	1.26-2.52	0.90	1.58±0.09
Urmoi	900	1.43	1.15-1.77	5.63	2.17±0.01
			48 HAT		
Karanja	900	1.79	1.22-2.62	2.10	1.49±0.09
Mahogany	900	1.50	1.16-1.93	5.94	1.99±0.01
Neem	900	1.19	0.96-1.48	2.29	1.55±0.08
Urmoi	900	0.97	0.86-1.09	7.62	2.14±0.08
			72 HAT		
Karanja	900	1.24	0.96-1.59	1.83	1.52±0.09
Mahogany	900	1.21	0.98-1.49	4.46	1.73±.09
Neem	900	0.91	0.78-1.06	1.51	1.61±0.08
Urmoi	900	0.72	0.66-0.79	12.16*	2.25±0.08

HAT= Hour after treatment.
Values were based on two plant parts, three solvents, five concentrations, three replications of 10 insects (5 ♂ and 5 ♀) each. χ^2= Goodness of fit. The tabulated value of χ^2 is 7.81 (d .f = 3 at 5 % level).

The probit regression lines became steeper as doses increased, because the adult insects were treated with more toxins for the same period at higher doses. The present study revealed the reduction of insect population due to use of plant extracts and agreed with the previous findings of Shahjahan *et al.* (2003), Rahman *et al.* (2004), Saha (2006), Begum *at al.* (2007), Khanam *et al.* (2008) and Mamun *et al.* (2009) .

CONCLUSIONS

Botanical pesticides are most appropriate for use in organic food production when it comes to agricultural pest management. We must import a significant amount of insecticides annually in order to reduce the serious harm that insect pests inflict in Bangladesh. Most of our farmers can no longer afford synthetic insecticides to protect their stored goods because of the high prices of insecticides. On the other hand, we have a long tradition of using locally accessible botanicals to preserve our stored grains. In many situations, aqueous extracts have shown themselves to be very effective against stored product insects, and the application of plant materials is easy. Additionally, compared to imported chemical insecticides, they are significantly less expensive and simpler to handle and prepare. Utilizing botanical elements as insecticides will be advantageous for our farming industry. They are not only of low cost, but also have no environmental impact in term of insecticidal hazard. The compounds of urmoi plant could be used for the development of novel botanical insecticides with highly precise targets for sustainable insect pest management. The plant materials used in this study are helpfull as protectant to reduce the pest attack in the storage significantly. Farmers may use neem fruit extracts for grain in seed storage and urmoi fruit extract can be used for the storage of seed grain and other agricultural plant protection purposes except storage of food grain.

Acknowledgements

The Department of Entomology at Bangladesh Agricultural University is deeply appreciated by the author(s) for providing all the facilities and assistance needed for this research effort. The author would like to thank the former and acting Executive Chairman of BARC for providing financial support, including a PhD fellowship through the BARC Manpower Development Program and the necessary research fund, without which the study would not have been possible.

Conflict of Interest

The authors declare that there is no conflict of interest among authors regarding the submission and publication of this manuscript.

DOI: https://doi.org/10.55706/jae1734

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DOI: https://doi.org/10.55706/jae1734